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(71)(72) Applicant and Inventor: KANKARE, Jouko [FI/FI]; Paljaspää 8 E, SF-20610 Turku (FI).

(74) Agent: HAKOLA, Unto; Tampereen Patenttitoimisto Oy, Kanslerinkatu 6, SF-33720 Tampere (FI).

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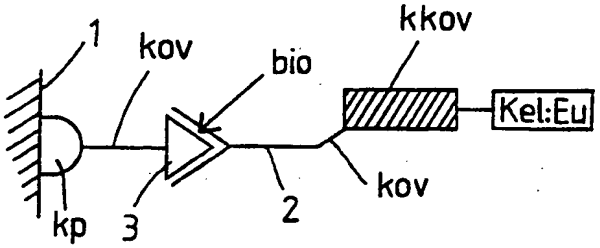

(57) Abstract

In the assay method based on a bioaffinity reaction on a solid phase (1), the measurement is performed by using time-resolved fluorometry with fluorescent lanthanide chelates. A single lanthanide chelate (Kel:Eu) is covalently bound (bond kkov) to one bioaffinity compound (2) of the bioaffinity reaction for producing a labelled compound. After the bioaffinity reaction and washing, the lanthanide chelate containing both the chelating structure and the lanthanide ion in chelated form is released from the solid phase (1) and measured from the solution by time-resolved fluorometry.

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Time resolved lanthanide chelate fluorometric assay.

1. Subject of the invention

The invention relates to a bioaffinity reaction performed on a solid phase. The bioaffinity reaction comprises the following assays: immunoassays, nucleic acid hybridizations, ligand-lectin assays. The method utilizes time-resolved fluorometry in connection with the bioaffinity reaction, the other partner taking part in the reaction being covalently labelled with a lanthanide chelate. When the bioaffinity reaction is completed, the lanthanide chelate is assayed by time-resolved fluorometry.

The invention covers different biospecific and bioaffinity-based reactive partners, although an immunoreaction is the most common type. The invention is thus primarily described as an immunochemical assay. The term refers to both competitive and non-competitive assay principles (R. Ekins et al. Pure & Appl. Chem 57 (1985) pp. 473-482), in which one of the reactive partners is labelled with a measurable group. Such immunochemical reagents that can be labelled with measurable groups include antibodies, antigens and haptens.

2. Methods of prior art related to the invention

In the last decade, lanthanide chelates have become generally accepted labelling agents in connection with time-resolved fluorometry e.g. in immunochemical methods (Lövgren et al., Luminescence Immunoassays and Molecular Applications, Ed. van Dyke, CRC-Press, 1990, pp. 233-253). From spectroscopic and quantum chemical data it has been inferred that in the lanthanide series, Dy^{3+}, Sm^{3+}, Tb^{3+} and Eu^{3+} are the

most suitable, because they emit delayed fluorescence. Taking into account the foreseeable advantages in practice, a finding that the lanthanide chelates show delayed fluorescence with a life time longer than 10 μs has generally been hoped for. Furthermore, the lanthanide chelates used should have a sufficiently high stability coefficient for being capable of binding a lanthanide ion efficiently during an immunochemical or some other bioaffinity reacion (Lövgren, Alternative Immunoassays (1985), pp. 203-217) without losing the good absorption of excitation energy by the chelate and for being capable of transferring excitation energy to the chelated lanthanide ion. Several different alternatives have been developed to avoid this problem, because chelates having both good chelating ability and good energy absorption and fluorescence properties have not been available.

By the most widely used hererogeneous time-resolved fluoroimmunoassay, the DELFIA^R assay (Wallac Oy, Turku, Finland), the stability problem has been solved by using a covalently bound non-fluorescent lanthanide chelate for labelling the partner taking part in the immunoreaction (hapten, antigen, antibody). In the DELFIA^R assay, the lanthanide ion is dissociated from the chelate ligand into the solution at pH 4 after the immunoreaction has been completed. The ion is separated from the ligand which is covalently bound to the immunochemical partner bound in the solid phase.

Attempts have been successful to make the lanthanide ion exhibit fluorescence in an aqueous solution, in a so-called developer solution containing (a) a surface-active agent, (b) a chelate compound, with which the lanthanide ion exhibits fluorescence, and (c) a synergistic compound. The intensity of the fluorescence and its half-life are dependent not only on the lanthanide but also on the pH value, on the surface-

active agent, on the synergistic compound and on the chelate compound (Halvarson et al., J. Chem. Phys. 41 (1964), pp. 157 and 2752, and Hemmilä et al., Anal. Biochem. (1984), pp. 335-343).

In addition to the assay above, only one commercial time-resolved immunoassay system using fluorescence and lanthanide chelates has been developed (Cyberfluor), using 1,10-phenanthroline-2,9-dicarboxylic acid and some of its new derivatives (Khosvari et al., Clin. Chem. 33 (1988), pp. 1994-1999). These structures can be covalently bound to proteins, and they form fluorescent chelates in the presence of lanthanide salts. However, the stability of these chelates is relatively low, and consequently, additional lanthanide salt must be involved before measuring the time-resolved fluorescence on a solid phase. Moreover, the assay is less sensitive in comparison with DELFIA^{R}.

A system which is to some extent similar to that above has been patented by Wieder (U.S. Patent 4,058,732), although no evidence or results on the performance of the system have been published. The compound concerned, the reaction partner of the immunoassay, is labelled by fluorescence probes of a rare earth metal complex, such as by benzoyl trifluoroacetone, and the labelled compound is excited on a solid phase, the time-resolved fluorescence being measured after a certain delay time (U.S. Patent 4,058,732, Fig. 1). Also in this case, the stability of the lanthanide chelate presents a difficult problem in the practical applications.

Several alternative lanthanide chelates developed later have good fluorescence properties and a sufficiently high stability coefficient for chelating a lanthanide ion and for retaining stability under most conditions for bioaffinity assay. All these chelates

or compounds can be covalently bound together with one compound taking part in the bioaffinity assay. For example in a competitive or non-competitive immunoassay on a solid phase, the time-resolved fluorescence is measured after the completion of the immunoassay directly from the immobilized labelled reaction partner. Alternatively, different homogeneous assays (no dissociation) can be developed for measuring by time-resolved fluorescence of either the change in the solution concentration of the labelled reaction partner (U.S. Patent 4,920,195 and European Patent 0203047) or the change in the fluorescence intensity and/or in the delay time of the lanthanide chelate used, which is influenced by the bioaffinity reaction between labelled and unlabelled reaction partners (U.S. Patent 4,587,223; European Patent Application 88850314.1).

The dissociation stage can be avoided by measuring the content of the compound bound in the solid phase selectively (European Patent Application 86300588.0).

In most of the methods described, the time-resolved fluorescence is measured either directly from the labelled compound on a solid phase or by using the principle of the dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA^R). In this method, the lanthanide ion as such is dissociated quickly at a low pH value, whereby a new, intensively fluorescent chelate is formed with the components of a so-called developer solution. Both alternatives work, although they have certain disadvantages.

If the time-resolved fluorescence is measured directly from the labelled bioaffinity compound on a solid phase, an intensely fluorescent stable lanthanide chelate is required. The optical requirements for the assay device are high, because the exciting light

must be directed on a limited surface used as the solid phase in the assay. Furthermore, during direct excitement, many solid surfaces emit considerable background fluorescence with a long half-life limiting the sensitivity of the assay.

The DELPHIA^{R} assay has none of the limitations mentioned above, because the lanthanide is measured from a solution in which it has formed a new intensively fluorescent chelate. However, because of the dissociation of the lanthanide ion from a solid phase, from the labelled compound, followed immediately by the development of a new chelate in the solution, the developing stage is very sensitive to external lanthanide contaminations. This will result in the immediate growth of "background" fluorescence (Diamandis, Clin. Biochem 21 (1988), pp. 139-150). The possibility of contamination is excluded by the use of lanthanide chelates which are both stable and fluorescent.

3. The Invention

The invention relates to time-resolved fluorometry and a fluorescence measurement from a lanthanide chelate label after a bioaffinity reaction taken place on a solid phase. The bioaffinity reaction comprises the following methods: immunoassays, nucleic acid hybridizations, ligand-lectin assays and ligand-receptor assays. After the bioaffinity reaction is completed on a solid phase, the lanthanide chelate is released into the solution from the bioaffinity complex bound to the solid phase, and it can be measured directly from the solution by time-resolved fluorometry.

4. Detailed Description of the Invention

The invention can be applied in several biospecific affinity reacions of different types, although the immunochemical reaction is the most common type. For this reason, the invention is primarily described as an immunochemical assay. The different biospecific affinity reactions that are feasible are illustrated in Figs. 1 to 5. The competitive reaction alternatives 1 to 3 are described in Figs. 1 to 3 and the non-competitive reaction alternatives 1 to 2 in Figs. 4 and 5. Both the competitive and the non-competitive principle of immunoassay are described above presuming that the immunoassay part of the reaction has been completed.

In Figs. 1 to 5, the following reference signs are used: solid phase 1, carrier protein kp, antibody 2, antigen or hapten 3, antigen 4, stable and fluorescent lanthanide chelate Kel:Eu, bioaffinity bond arrow bio, covalent bond kov, and cleavable covalent bond between the lanthanide chelate and the bioaffinity compound kkov.

According to the invention, the lanthanide chelate must be released in the solution. This can be performed either by cleaving one of the bioaffinity bonds described or by breaking the covalent bond or by combining these two alternatives. Using a method in which only the bioaffinity bonds are broken, no cleavable covalent bond is needed between the fluorescent lanthanide chelate and the labelled bioaffinity compound. During each releasing process, the chelate complex between the lanthanide ion and the chelate ligand must remain intact. Thus only the fluorescent lanthanide chelate is released into the solution, in which it can be measured effectively by time-resolved fluorometry. Therefore, no background induced by the

material of the solid phase nor any external lanthanide contamination can interfere with the result.

The bioaffinity bond between the reagents (antibodies, haptens, antigens, nucleic acids, receptors, lectins, carbohydrates, hormones, etc.) is mainly composed of hydrophobic interactions, hydrogen bonds, Van der Waals forces and ionic interaction. Therefore, these bonds must be broken for releasing the fluorescent lanthanide chelate into the solution. Several factors are known for breaking bioaffinity bonds, such as the pH value, ion strength, caotropic salts, detergents, organic solvents, etc. These factors can be used for breaking the bioaffinity bond to release the fluorescent lanthanide chelate into the solution.

The use of breakable bifunctional molecules and bonds is known for detecting the contact sites of biomacromolecules, and they have also been successfully used in affinity chromatography for elution of very strongly bound ligands (Jayabaskaran et al., Prep. Biochem. 1987, 17, pp. 121-141; Montan et al., Arch. Biochem. Biophys. 1982, 218, pp. 101-108; Singh et al., Arch. Biochem. Biophys. 1979, 193, pp. 284-293; Herman et al., Anal. Biochem. 1986, 156, pp. 48-55), for purification of macromolecular reagents (Schwarzberg, U.S. Patent 4,272,506), for formation of bidirectional synthetic vesicles (Chang et al., Chem. Lett. 1987, pp. 1385-1388), for bidirectional immobilization of enzymes (Carlsen, Hind. Antibiot. Bull. 1978, pp. 105-108), and for determination of the chemical state of ligand titres and the solid phase (Marburg et al., Anal. Biochem. 1989, 181, pp. 242-249). The prior art includes also a patented reagent (British Patent 1,597,758) that is used in assays based on biospecific affinity reactions. The reagent comprises a labelled immunochemical component composed of a multiconjugate with several analytically detectable

groups connected to each other by breakable covalent bonds. The said multiconjugate, in turn, is bound by several breakable bonds to the immunochemical component.

According to our knowledge, single covalently breakable bonds have not been previously used for releasing a single label from the labelled bioaffinity compound to the solution, after the bioaffinity reaction has been completed in the solid phase. Furthermore, it is not known that breaking a single covalent bond to release a single label be used simultaneously in connection with breaking bioaffinity bonds. Moreover, breaking affinity bonds for dissolving the label into the solution after a bioaffinity reaction that has taken place on a solid phase has not been previously used.

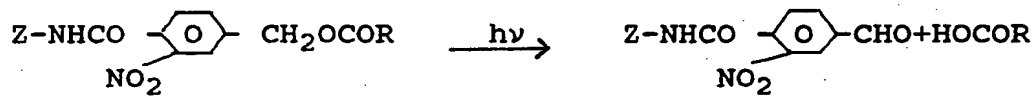
The most common covalent bonds and the methods used for breaking them are listed below:

Breakable bond	Chemical breaking method
Disulphide	Reduction
Vicinal glycols	Periodates
Phenyl ester	Base / hydroxylamine
1-oxidized sulphone	Base
Azo	Dithionite
bis-glycol ester	Hydroxylamine
Thioester	Hydroxylamine

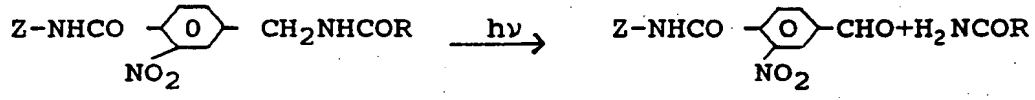
In addition to chemical methods, also exposure to light can be used for breaking photosensitive bonds (Chow, The Chemistry of Amino, Nitraso and Nitro Compounds and Their Derivatives, Part 1, Ed. Patai, J. Wiley & Sons, N.Y. 1982, pp. 181-287).

O-nitrophenyl groups are typical examples of bonds breakable by light:

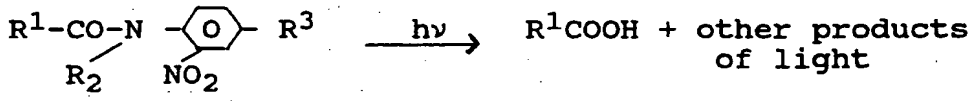
D.H. Rich & S.K. Gurwara, J. Am. Chem. Soc. 97 (1975) 1575:



D.H. Rich & S.K. Gurwara, Tetrahedron Lett. 1975 301:



B. Amit & Patchornik, Tetrahedron Lett. 1973 2205:



Several alternative covalent bonds and chemical breaking methods, also including light, can be used for breaking a covalent bond so that the fluorescent lanthanide chelate is released into the solution.

Similar methods can be applied also to bioaffinity assays other than immunochemical assays. For example in nucleic acid hybridization assays on a solid phase, the label can be released into the solution, when the hybridization assay has been completed. Figs. 6 and 7 show the typical assay alternatives; only the release of the fluorescent lanthanide chelate into the solution is not presented. In addition to the reference signs used in Figs. 1 to 5, the following signs have been used: immobilized probe 5, target DNA 6, labelled probe 7, biotinylated probe 8, biotin bt, and immobilized streptavidin 9.

Also in hybridization assays, the fluorescent lanthanide chelate can be released into the solution either by breaking one of the visible bioaffinity bonds or by breaking a covalent bond or by combining these two alternatives. During the releasing process, the chelate complex between the lanthanide ion and the chelate ligand is kept intact.

The invention can be described in more detail by the following non-restrictive examples:

EXAMPLE 1

Breaking an immunoaffinity bond in a non-competitive hTSH-assay

The experiment is arranged in a way similar to a non-competitive assay (Fig. 4) except that the covalent bond is excluded.

Assay

Polystyrene microtitre wells were coated with a monoclonal anti-hTSH antibody and the surface was impregnated with BSA. Another monoclonal anti-hTSH antibody was labelled with fluorescent europium chelate, namely with an isothiocyanate derivative of 4-aminophenyl-ethynyl-2,6-bis(N,N-bis(carboxymethyl)-aminomethyl)pyridine-Eu. This assay was performed in a single stage in the coated microtitre wells containing 50 µl of determination buffer, hTSH standard, and 50 µl of the labelled antibody (50 ng). Incubation was carried out at room temperature (30 min) during continuous shaking of the microtitre plate. The wells were sucked dry and washed six times, after which the bioaffinity bond was broken by adding 200 µl various breaking solutions. The wells were shaken for 2 min, whereafter the time-resolved fluorescence was measured

from each breaking solution at intervals of 2, 7, 32, 68 min. The composition of the breaking solutions was the following: each contained 50 mM carbonate buffer with a pH value of 10.0 and 0.1% BSA and either

0.2	Triton X-100 or
20%	Ethanol or
10%	Glycerol or
1.0%	Acetone or
10%	Propanol or
5 mM	NaSCN

The signal level of the highest standard (500 µIU/ml) and its stability are shown in Fig. 11. In each case, a complete standard diagram was the aim. Fig. 8 shows the standard curve on the use of 20% ethanol.

EXAMPLE 2

Breaking a breakable covalent bond

In the model assay, the fluorescence signal was solely dependent on breaking the covalent bond, because no bioaffinity bond was involved.

Assay procedure

Polystyrene microtitre wells were coated with 1 µg of BSA (200 µl) which was labelled with a europium chelate derivative of isothiocyanate disulphide (SCN-S-S) (Example 1). Breaking solution (200 µl) was added to the coated wells, and after shaking for 2 min, the time-resolved fluorescence of the europium chelate in the solution was measured. Several breaking solutions were tested which had been prepared into a 50 mM carbonate buffer.

Breaking solution	pH	Dithiotreitol mM	EtOH %	BSA %
1	9	2	-	-
2	9	5	5	0.1
3	9	10	10	0.5
4	10	2	5	0.5
5	10	5	10	-
6	10	10	-	0.1
7	11	2	10	0.1
8	11	5	-	0.5

It is substantial that no BSA was released from the solid phase during breaking. Fig. 12 shows the fluorescence measured from the solution in the conditions described above after a breaking time for 2 and 53 minutes.

EXAMPLE 3

Simultaneous breaking of immunoaffinity bonds and a breakable covalent bond in a non-competitive hTSH assay

The test was carried out in a manner analogous to the non-competitive assay presented in alternative 1.

Assay procedure

Polystyrene microtitre wells were coated with a monoclonal anti-hTSH antibody, and the surface was impregnated with BSA. Another monoclonal anti-hTSH antibody was labelled with a fluorescent europium chelate which is an isothiocyanate disulphide derivative of 4-aminophenylethynyl-2,6-bis-(N,N-bis(carboxymethyl)-aminomethyl)pyridine-Eu. During the immunoassay (30 min at room temperature, continuous shaking), the wells contained 50 µl of hTSH standard (0, 1, 10, 50,

250 and 500 μIU/ml) and assay buffer (50 μl) containing the labelled antibody (50 ng). After incubation, the wells were sucked dry and washed six times before adding the breaking solution (200 μl). The solution contained 20% EtOH, 2 mM dithiotreitol and 0.1% BSA in 50 mM carbonate buffer, pH 10. After shaking for 2 minutes, the time-resolved fluorescence of the europium chelate in the solution was measured. The results are given in Fig. 9.

EXAMPLE 4

Simultaneous breaking of immunoaffinity bonds and a breakable covalent bond in a competitive assay of 17-α-hydroxyprogesterone

The test procedure is described in connection with the competitive assay in Fig. 3.

Assay procedure

Polystyrene microtitre wells are coated with polyclonal anti-rabbit antibody. A polyclonal rabbit anti-17-α-OH-progesterone antibody was diluted in the assay buffer (1:50,000), and 100 μl of the dilution was added into each well. Thereafter, 25 μl of standard was added (7, 16, 28, 57, 102 and 261 nM). Finally at stage 3, a 17-α-OH-progesterone derivative, labelled with an isothiocyanate disulphide derivative of 4-aminophenyl-ethynyl-2,6-bis(N,N-bis(carboxymethyl)-aminomethyl)-pyridine-Eu, was added (100 μl, 4.8 nM). Incubation was performed for 3 hours at room temperature by continuous shaking. Next, the wells were sucked dry and washed six times, whereafter 200 μl of the breaking solution was added (as in Example 3) and the time-resolved fluorescence of the europium chelate in the solution was measured after 2 minutes had elapsed

from shaking. Fig. 10 shows the standard diagram of 17-α-OH-progesterone assay. The maximum signal obtained for the 0-standard was 55,000 cps.

Claims:

1. An assay method based on a bioaffinity reaction on a solid phase, in which method the measurement is performed by using fluorometry, particularly time-resolved fluorometry, by means of fluorescent lanthanide chelates, whereby a single lanthanide chelate is covanlently bound to one bioaffinity compound of the bioaffinity reaction for producing a labelled compound, and the excess of the labelled compound is washed away after the completion of the bioaffinity reaction, **characterized** in that subsequent to the bioaffinity reaction and the wash, the lanthanide chelate, containing both the chelating structure and the lanthanide ion in the chelated form, is released from the solid phase and measured from the solution by means of fluorometry, particularly time-resolved fluorometry.

2. Method according to Claim 1, **characterized** in that upon the release of the lanthanide chelate from the solid phase, it is still covalently bound to the said one bioaffinity compound of the bioaffinity reaction.

3. Method according to Claim 2, **characterized** in that after the release, the same lanthanide chelate is assayed from the solution by using fluorometry, particularly time-resolved fluorometry, the chelate being still bound to the said bioaffinity compound.

4. Method according to Claim 2 or 3, **characterized** in that the lanthanide chelate is released from the solid phase by breaking the bond between the bioaffinity compounds on the solid phase e.g. by one or several of the following factors: pH, temperature, detergent, caotropic salt, organic diluent, and ion strength.

5. Method according to Claim 1, characterized in that a single lanthanide chelate is released from the labelled bioaffinity compound bound to the solid phase by breaking the breakable covalent bond between the labelled compound and the chelate.

6. Method according to Claim 5, characterized in that the breakable covalent bond which is broken between the labelled bioaffinity compound and the single lanthanide chelate is a disulphide bond (-S-S-), a vicinal diol, an ester bond, a diselenium bond, or a photosensitive bond, or a combination thereof.

7. Method according to Claim 6, characterized in that the covalent bond between the labelled bioaffinity compound and the single lanthanide chelate is broken by reduction, by oxygenating hydrolysis, or by light, or by a combination thereof.

8. Method according to Claim 1, characterized in that the lanthanide chelate containing both the chelating structure and the lanthanide ion is released from the bioaffinity complex bound to the solid phase by breaking both the bioaffinity bond between the reacted bioaffinity compounds and the breakable covalent bond between the labelled bioaffinity bond and the single chelate, whereby the bioaffinity bond is broken e.g. by one or several of the following factors: pH, temperature, detergent, caotropic salt, organic diluent, and ion strength, and the breakable covalent bond is broken e.g. by reduction, by oxygenating hydrolysis, or by light, or by a combination thereof.

9. Method according to Claim 5 or 8, characterized in that after the release, the same lanthanide chelate is measured from the solution by using fluorometry, particularly time-resolved fluorometry.

10. Method according to one of the Claims 1 to 9 above, characterized in that the bioaffinity reaction is an antigen-antibody reaction, a nucleic acid hybridization reaction, a ligand-receptor reaction, or a ligand-lectin reaction.

11. Method according to one of the Claims 1 to 10 above, characterized in that the chelated lanthanide ion is either europium, terbium, samarium, or dysprosium.

FIG.1

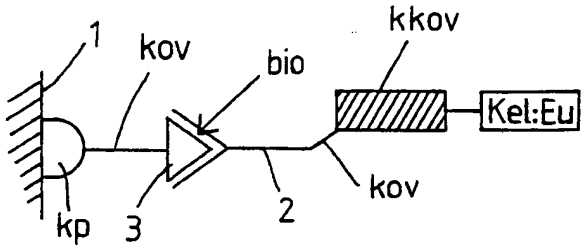


FIG.2

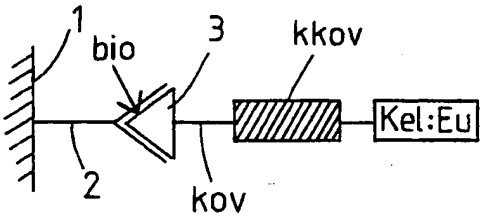


FIG.3

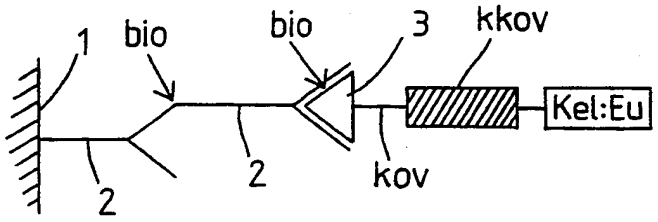


FIG.4

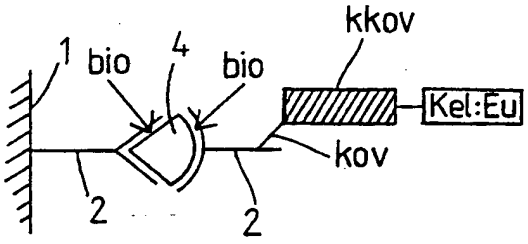
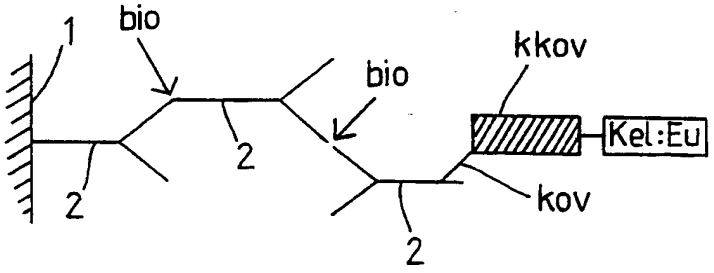
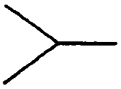


FIG.5



Definitions for Figs. 1 to 5

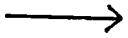

= carrier protein (kp)


= antibody (2)


= antigen or hapten (3)


= antigen (4)


= breaking covalent bond (kk)


= bioaffinity bond (bio)

= stable and fluorescent lanthanide chelate

= covalent bond (kov)

FIG.6

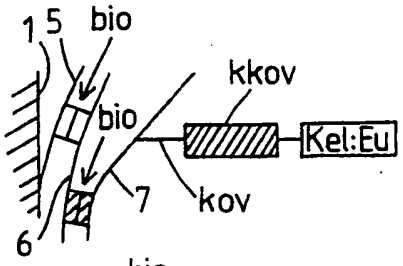
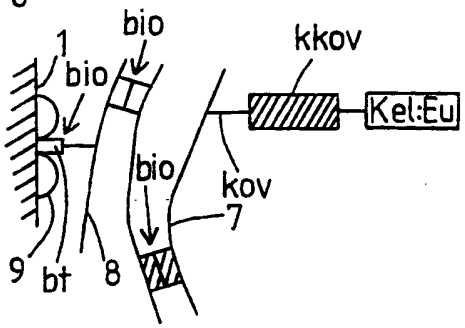


FIG.7



Definitions:

= immobilized probe (5)

= target DNA (6)

= labelled probe (7)

= biotinylated probe (8)

= biotin (bt)

= immobilized streptavidine (9)

= breakable covalent bond (kkov)

Kel:Eu = stable and fluorescent lanthanide chelate

= bioaffinity bond (bio)

= covalent bond (kov)

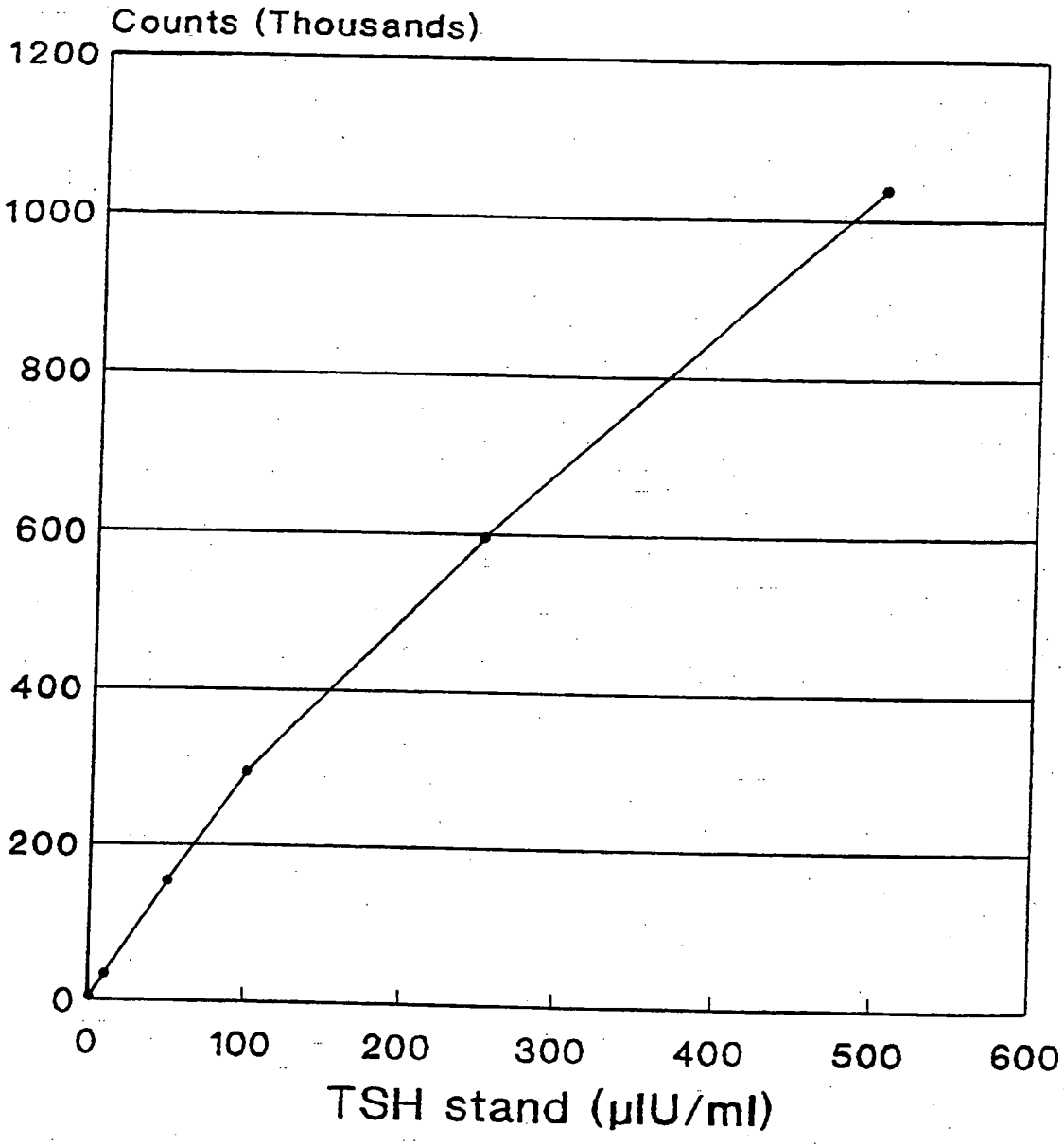

Counts (Thousands)
1200
1000
800
600
400
200
0
0
100
200
300
400
500
600
TSH stand (µIU/ml)

FIG.8

TSH-ASSAY

Measurement solution: 50 mM carb. pH 10,
20 % EtOH, 2mM DTT, 0.1% BSA

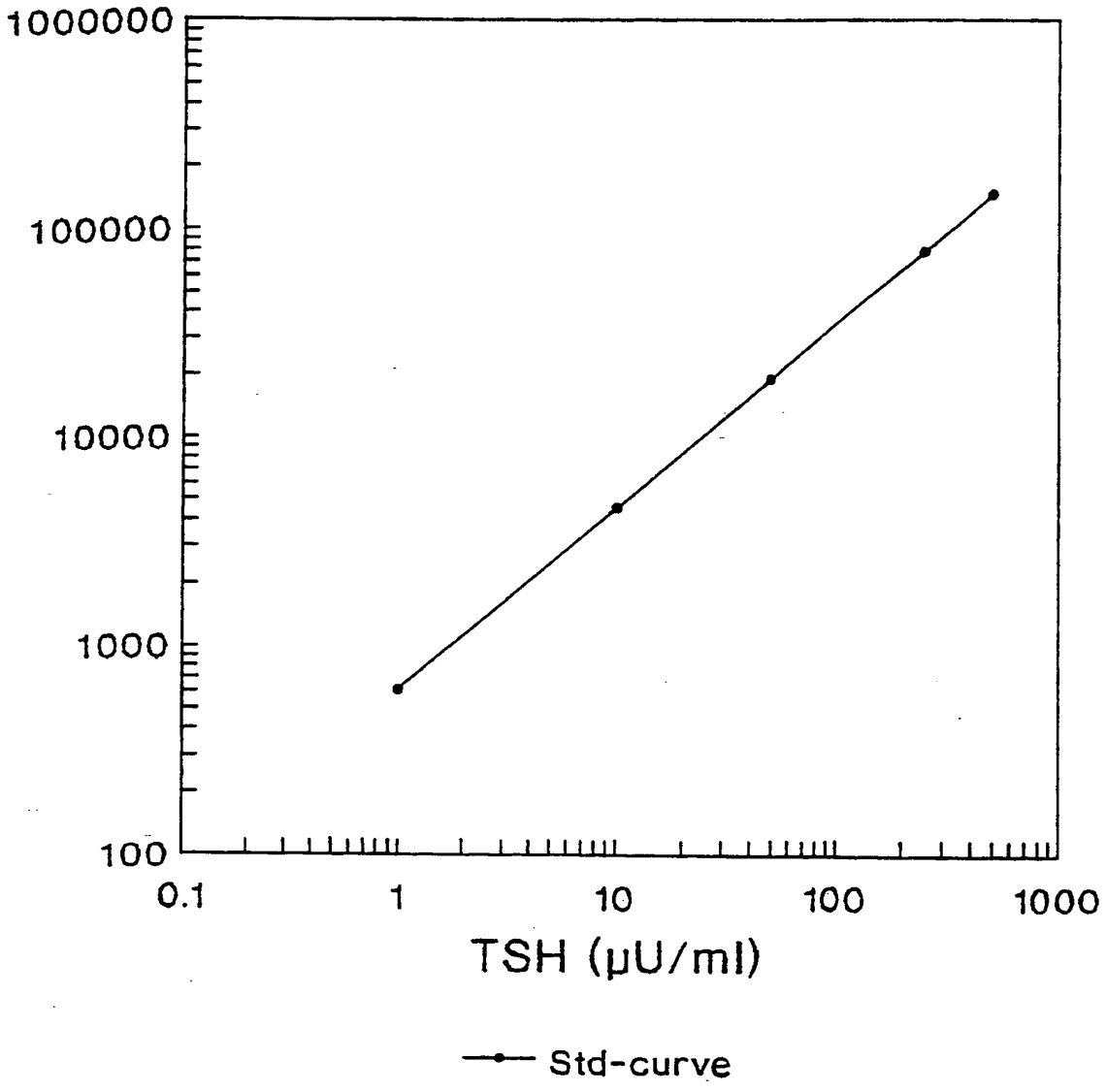


FIG.9

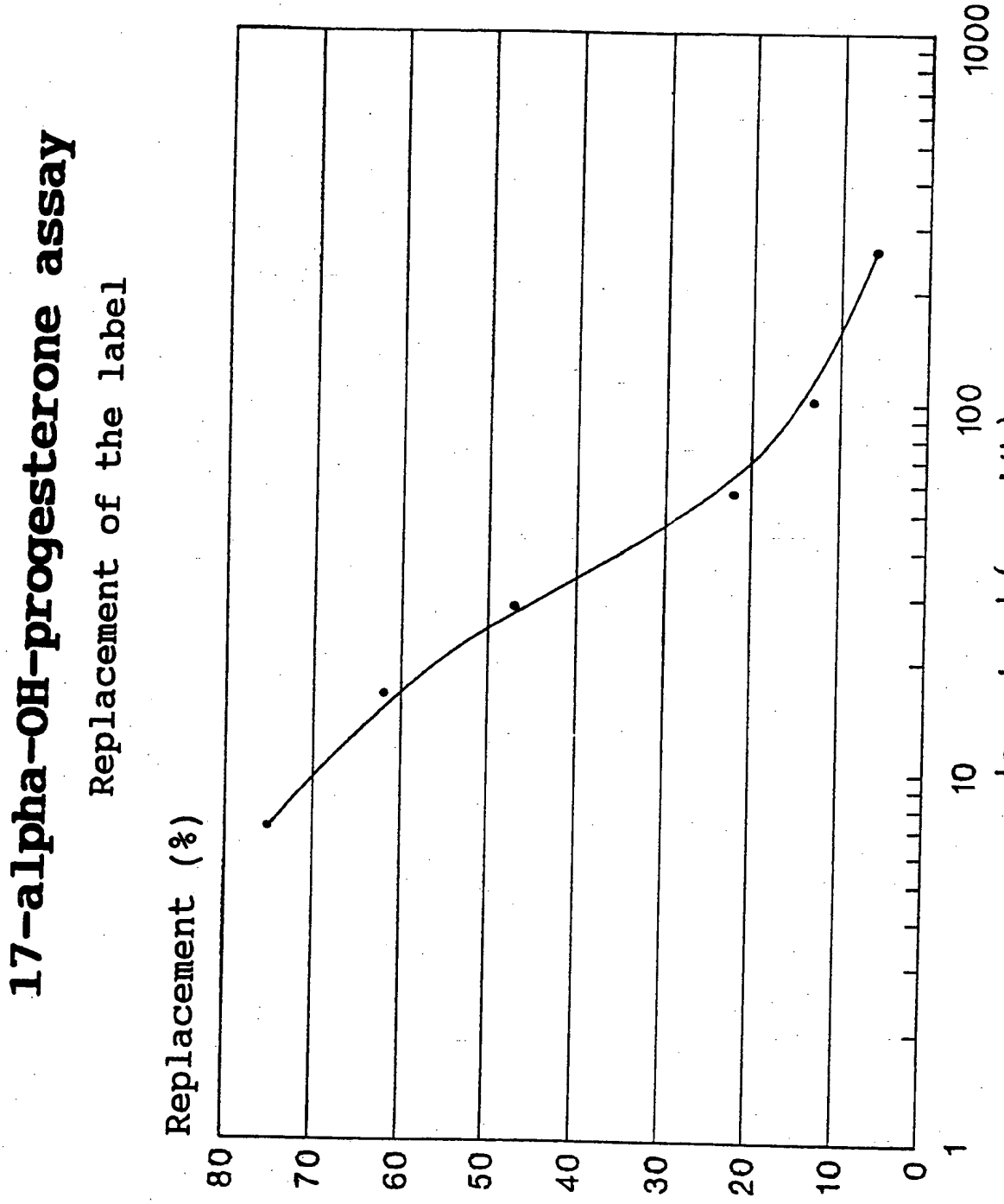

17-alpha-OH-progesterone assay
Replacement of the label
Replacement (%)
80
70
60
50
40
30
20
10
0
1
10
100
1000
log stand (nmol/L)

FIG.10

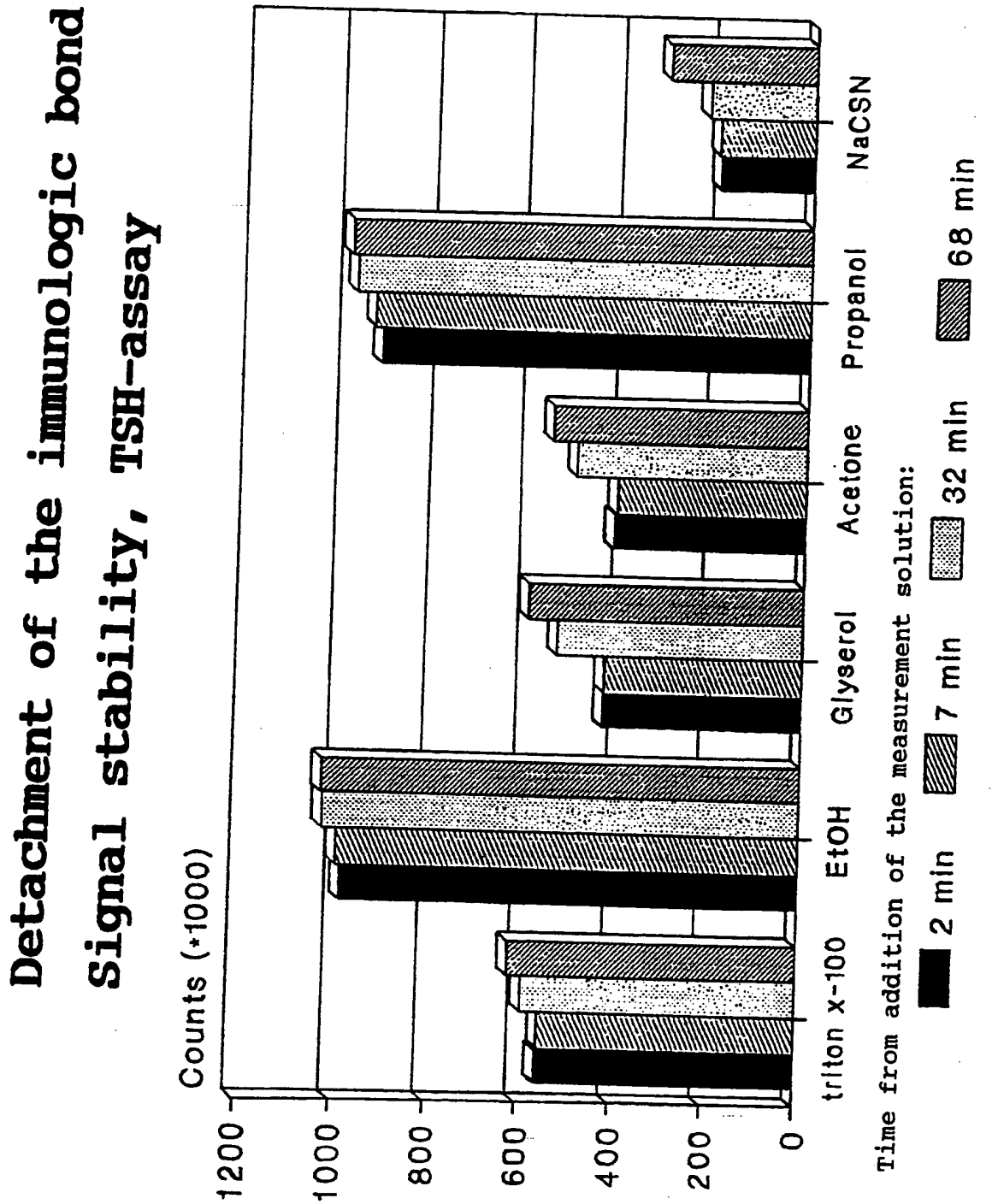

Detachment of the immunologic bond
Signal stability, TSH-assay
Counts (*1000)
1200
1000
800
600
400
200
0
triton x-100
EtOH
Glyserol
Acetone
Propanol
NaCSN
Time from addition of the measurement solution:
2 min
7 min
32 min
68 min

FIG.11

Reduction of the sulphur bridge Signal and stability

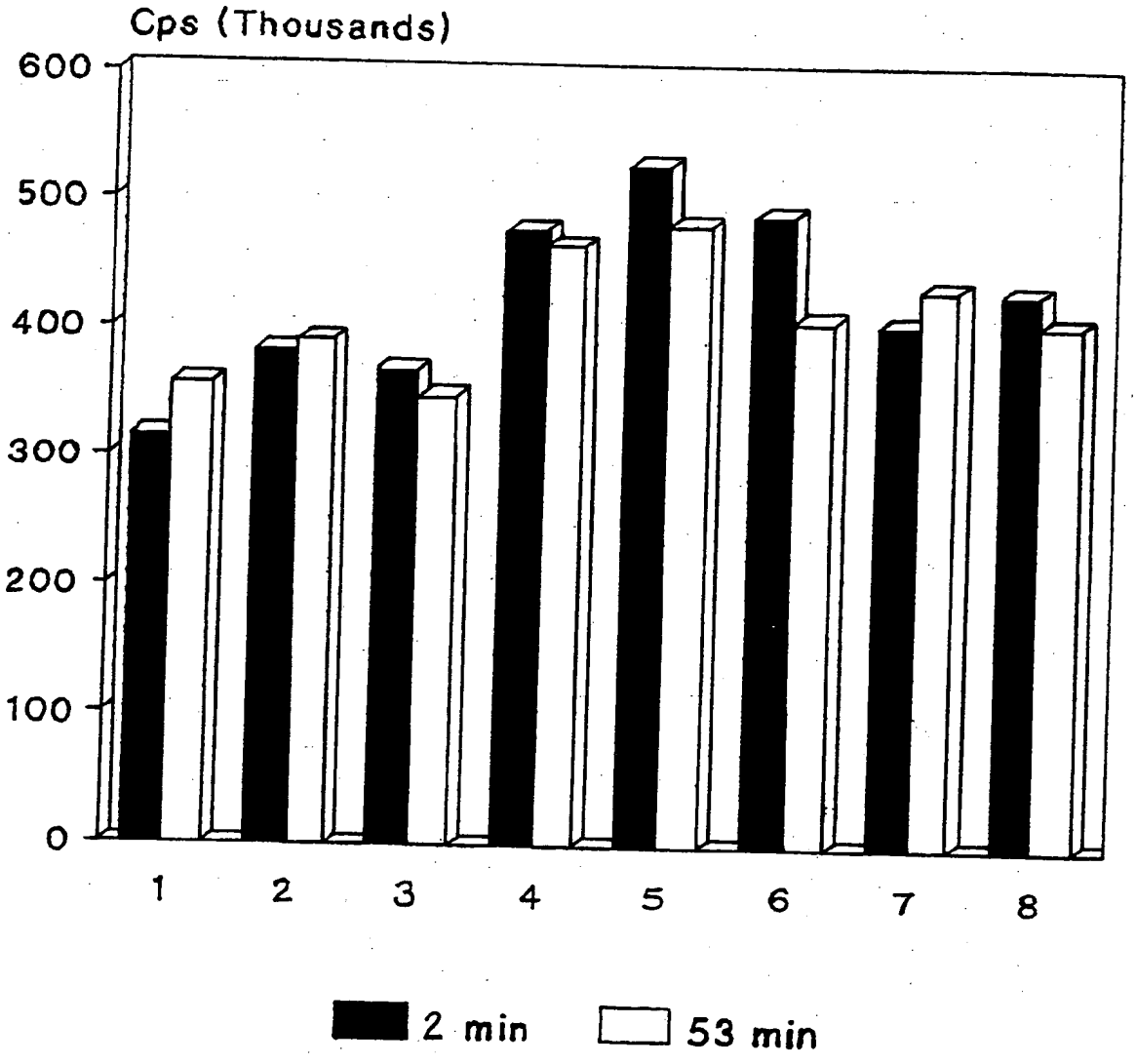


FIG.12

INTERNATIONAL SEARCH REPORT

International Application No PCT/FI 92/00068

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: G 01 N 33/543, 533, C 12 Q 1/68

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	G 01 N; C 12 Q

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in Fields Searched[8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
Y	EP, A1, 0242095 (SCLAVO, INC.) 21 October 1987, see the whole document --	1-11
Y	US, A, 4576912 (YAVERBAUM ET AL) 18 March 1986, see the whole document --	1-11
A	US, A, 4650750 (GIESE) 17 March 1987, see the whole document --	1-11
A	US, A, 4891324 (PEASE ET AL) 2 January 1990, see column 10 - column 12; claims 1,18 --	1-11

* Special categories of cited documents:[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
15th June 1992	1992 -06- 17
International Searching Authority	**Signature of Authorized Officer**
SWEDISH PATENT OFFICE	Carl-Olof Gustafsson

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	ANALYTICAL BIOCHEMISTRY, Vol. 181, 1989 Stephen Marburg et al: "Chemistry on solid supports: Defining events and titers by use of cleavable, assayable linking molecules", see page 242 - page 249 --	1-11
Y	WO, A1, 8802489 (EKINS, ROGER, PHILIP) 7 April 1988, see the whole document --	1-11
Y	EP, A1, 0159066 (WALLAC OY) 23 October 1985, see the whole document --	1-11
Y	ANALYTICAL CHEMISTRY, Vol. 62, No. 22, 1990 Eleftherios P. Diamandis et al: "Europium chelate labels in time-resolved fluorescence immunoassays and DNA hybridiation assays", see page 1149 - page 1157 especially page 1152A and 1154A-1155A --	1-11

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.PCT/FI 92/00068

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the Swedish Patent Office EDP file on 29/05/92
The Swedish Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A1- 0242095	87-10-21	JP-A-	63024155	88-02-01
		US-A-	4780421	88-10-25
US-A- 4576912	86-03-18	CA-A-	1124643	82-06-01
US-A- 4650750	87-03-17	CA-A-	1246058	88-12-06
		EP-A-	0085554	83-08-10
		JP-A-	58146540	83-09-01
		US-A-	4709016	87-11-24
US-A- 4891324	90-01-02	EP-A-B-	0275139	88-07-20
		JP-A-	63191065	88-08-08
WO-A1- 8802489	88-04-07	EP-A-	0324759	89-07-26
EP-A1- 0159066	85-10-23	EP-A-B-	0064484	82-11-10
		JP-A-	57186170	82-11-16
		SE-B-C-	425439	82-09-27
		US-A-	4565790	86-01-21